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Note

Pharmacokinetic evaluation of indomethacin ethyl ester-loaded nanoencapsules

Vitória B. Cattani [a], Adriana R. Pohlmann [a,b], Teresa Dalla Costa [a,*]

[a] *Programa de Pós-Graduação em Ciências Farmacêuticas, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, Av. Ipiranga 2752, Porto Alegre, RS 90610-000, Brazil*
[b] *Instituto de Química, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil*

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ABSTRACT

Goals were to evaluate indomethacin ethyl ester-nanoencapsules (IndOEt-NC) pharmacokinetics in rats and the *in vivo* ester conversion to indomethacin (IndOH). After i.v. and oral administration exclusively IndOH was detected in plasma. The $AUC_{IndOEt\text{-}NC}/AUC_{IndOH}$ ratio after i.v. dosing was 0.68, accounting for dose and molecular weight differences, probably due to increased IndOH clearance after IndOEt-NC administration ($\alpha = 0.05$). The results confirm that antiedematogenic activity reported for IndOEt-NC is due to IndOH. Encapsulation did not protect the ester which *in vivo* is rapidly released and converted to IndOH, acting as a pro-drug.

Non-steroidal anti-inflammatory drugs, such as indomethacin (IndOH), present important renal and gastric side effects that limit their use (Vane and Botting, 1998). The production of pro-drugs (Bonina et al., 1997) and COX-2 selective inhibitors (Kalgutkar et al., 2000), as well as IndOH nanoencapsulation have been proposed to reduce the side effects of these drug (Raffin et al., 2003).

Combining those strategies, indomethacin ethyl ester (IndOEt) was entrapped within nanocapsules (IndOEt-NC), and the ester release profile in digestive simulated fluids demonstrated that nanocapsules simultaneously entrapped and protected the drug *in vitro*. Moreover, in paw edema assay, an antiedematogenic activity of IndOEt-NC after oral administration was observed (71% edema inhibition) (Cruz et al., 2006a,b). These results prompted us to evaluate the pharmacokinetics of the nanoencapsulated IndOEt to determine if the pharmacological response observed was due to the ester activity or to indomethacin formed *in vivo*.

IndOEt-NC were prepared by interfacial deposition of preformed polymers using poly(ε-caprolactone) (MW 65,000) (Aldrich, France), polissorbate 80, sorbitan monostearate (Delaware, Brazil), and capric/caprylic triglyceride (Brasquim, Brazil). The nanocapsules presented IndOEt content of 1.04 ± 0.09 mg/mL (HPLC), pH of 5.62 ± 0.45, mean particle size of 267 ± 31 nm and polydispersity lower than 0.2 (Zetasizer nano-ZS ZEN 3600, Malvern).

The animal studies (UFRGS Ethics in Research Committee, protocol 2005478) were performed on male Wistar rats (270–310 g). Animals that received the formulations by oral route were kept fasten overnight, with free access to water before drug administration.

Pharmacokinetic evaluation was performed after oral ($n = 7$) or i.v. administration ($n = 9$) of IndOH 10 mg/kg and IndOEt-NC 10 mg/kg oral ($n = 8$) or 5 mg/kg i.v. ($n = 5$) dosing. Oral IndOH was given as aqueous suspension (1 mg/mL, 1% of polysorbate 80) and intravenously as 5% glucose suspension (2 mg/mL, 6% of polysorbate 80). After administration, 200 μL of blood were harvested up to 24 h, centrifuged (14811 g/15 min at 21 °C) and the plasma was frozen at −20 °C until HPLC analysis.

IndOH and IndOEt were assayed in plasma using a HPLC/UV validated method. The HPLC system consisted of a Waters® 600 solvent delivery system, a 717 Plus auto-injector and UV 2487 detector. Chromatographic separation was achieved on a Nova-Pack C_{18} column (Waters®), at 45 °C, using acetonitrile:0.02 M ammonium dihydrogen phosphate (70:30, v/v), apparent pH 5, as mobile phase. 50 μL sample were injected and both drugs were detected at 267 nm.

Individual plasma profiles were evaluated by non-compartmental (Excel® 2003, Microsoft®) and compartment pharmacokinetic approaches (Scientist® 2.0, MicroMath®). Model selection was guided by visual inspection, model selection criteria (MSC) and correlation coefficient given by the software. Statistical analysis was performed by ANOVA followed by Tukey test ($\alpha = 0.05$).

IndOH individual plasma profiles after intravenous administration (10 mg/kg) were described by two compartment open model, employing weighed non-linear regression (1/concentration). The mean plasma is shown in Fig. 1A. The pharmacokinetic parameters determined (Table 1) are in agreement with those

* Corresponding author. Tel.: +55 51 3308 5418; fax: +55 51 3308 5437.
E-mail address: teresadc@farmacia.ufrgs.br (T.D. Costa).

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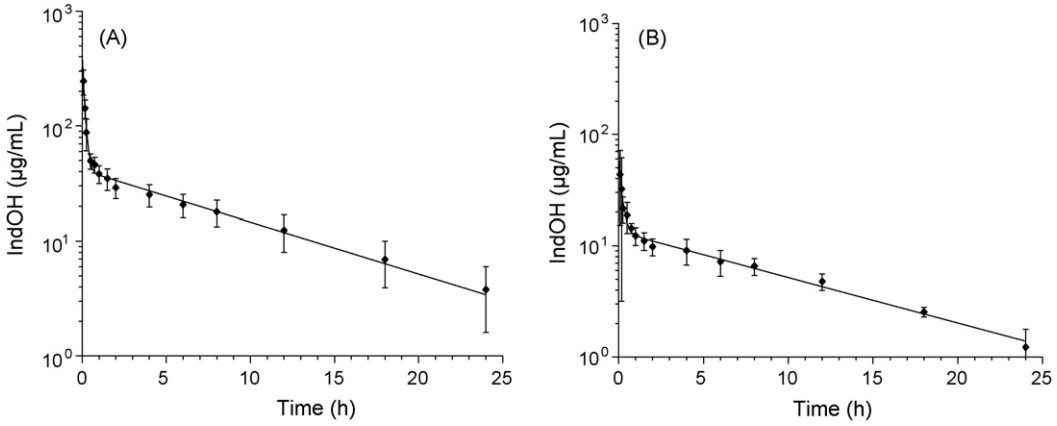

Fig. 1. IndOH plasma profiles after intravenous administration to Wistar rats: (A) IndOH 10 mg/kg ($n = 9$); (B) IndOEt-NC 5 mg/kg ($n = 5$) (average ± S.D.).

Table 1
Pharmacokinetic parameters determined after IndOH 10 mg/kg ($n = 9$) and IndOEt-NC 5 mg/kg ($n = 5$) intravenous administration to Wistar rats (average ± S.D.)

Pharmacokinetic parameters	IndOH 10 mg/kg		IndOEt-NC 5 mg/kg	
	Model independent	Two compartment	Model independent	Two compartment
A (μg/mL)	–	421.1 ± 161.2	–	75.3 ± 61.5[a]
B (μg/mL)	–	41.4 ± 6.4	–	13.3 ± 3.2[a]
α (h^{-1})	–	8.4 ± 2.6	–	8.7 ± 8.0
β (h^{-1})	0.11 ± 0.04	0.11 ± 0.03	0.12 ± 0.04[b]	0.10 ± 0.01
k_{21} (h^{-1})	–	0.89 ± 0.25	–	1.4 ± 0.8
k_{10} (h^{-1})	–	1.11 ± 0.46	–	0.55 ± 0.35[a]
k_{12} (h^{-1})	–	82.1 ± 50.7	–	55.5 ± 70.3
$ASC_{0-\infty}$ (μg h/mL)	475 ± 109	450 ± 112	151 ± 30	147 ± 29[a]
Vd_{SS} (L/kg)	0.18 ± 0.03	0.19 ± 0.03	0.29 ± 0.04	0.32 ± 0.09[a]
Cl_{TOT} (mL/(min kg))	0.37 ± 0.09	0.39 ± 0.10	0.53 ± 0.12	0.54 ± 0.12[a]
$t_{(1/2)\alpha}$ (h)	–	0.09 ± 0.04	–	0.20 ± 0.20
$t_{(1/2)\beta}$ (h)	6.8 ± 2.0	6.75 ± 1.90	6.3 ± 2.4	7.3 ± 0.7

[a] Significantly different of IndOH by compartmental approach ($\alpha = 0.05$).
[b] Significantly different of IndOH by model independent approach ($\alpha = 0.05$).

previously reported after IndOH 12 mg/kg i.v. administration to rats (Palakurthi et al., 2005).

After IndOEt-NC (5 mg/kg) intravenous administration, exclusively IndOH was detected in plasma (Fig. 1B), probably due to fast ester hydrolysis, besides drug nanoencapsulation. Thus, it was not possible to determine IndOEt conversion rate to IndOH with the sampling scheme used. A two compartment open model also described the individual IndOH concentration–time profiles after IndOEt-NC i.v. dosing. The resulting $AUC_{IndOEt\text{-}NC}/AUC_{IndOH}$ ratio was 0.68, already accounting for dose and molecular weight differences, probably due to a significant increase in total clearance after IndOEt-NC administration (Table 1).

The higher IndOH Cl_{TOT} after IndOEt-NC administration could be due to a fast nanocapsules uptake by the mononuclear phagocytic system or to central nervous system targeting caused by polysorbate 80 nanoparticles covering (Hans and Lowman, 2002). The

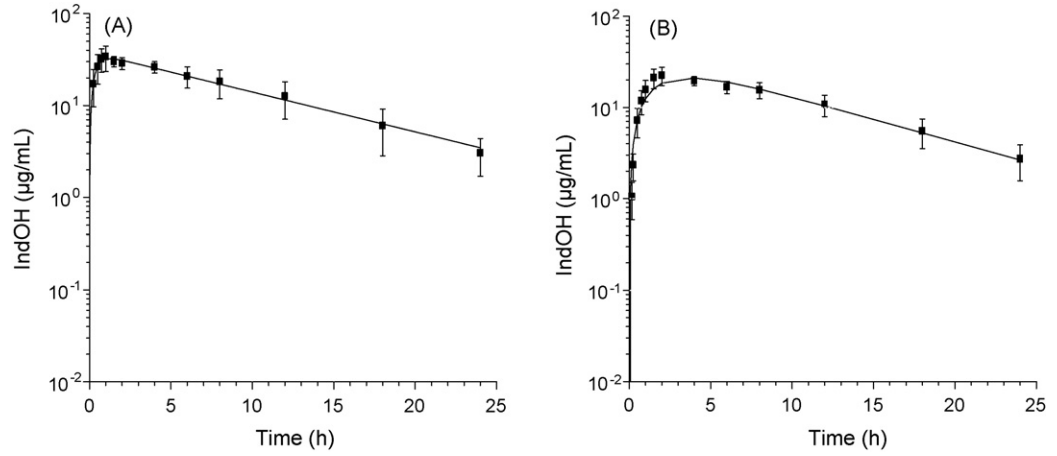

Fig. 2. IndOH plasma profiles after oral administration of 10 mg/kg to Wistar rats: (A) IndOH ($n = 7$); (B) IndOEt-NC ($n = 8$) (average ± S.D.).

Table 2
Pharmacokinetic parameters determined after IndOH 10 mg/kg ($n = 7$) and IndOEt-NC 10 mg/kg ($n = 8$) oral administration to Wistar rats (average ± S.D.)

Pharmacokinetic parameters	IndOH 10 mg/kg		IndOEt-NC 10 mg/kg	
	Model independent	One compartment	Model independent	One compartment
k_a (h^{-1})	–	2.8 ± 1.4	–	0.50 ± 0.22[a]
k_e (h^{-1})	0.12 ± 0.01	0.11 ± 0.04	0.12 ± 0.03	0.13 ± 0.04
$t_{(1/2)}$ (h)	5.7 ± 0.7	6.7 ± 2.3	6.1 ± 1.4	5.5 ± 1.5
$ASC_{0-\infty}$ (μg h/mL)	365 ± 97	366 ± 109	289 ± 54[b]	259 ± 53[a]
Cl_{TOT} (mL/min/kg)	0.38 ± 0.11	0.38 ± 0.13	0.36 ± 0.06	0.41 ± 0.08
Vd_{SS} (L/kg)	–	0.20 ± 0.03	–	0.19 ± 0.05
Cp_{max} (μg/mL)	35.8 ± 9.1	32.7 ± 5.6	24.2 ± 3.5[b]	19.5 ± 1.9[a]
t_{max} (h)	1.2 ± 0.6	1.4 ± 0.5	2.2 ± 0.8[b]	3.9 ± 0.9[a]
f (%)	77[c]	–	65[c]	–

[a] Significantly different of IndOH by compartmental approach ($\alpha = 0.05$).
[b] Significantly different of IndOH by model independent approach ($\alpha = 0.05$).
[c] Determined by AUC ration ($AUC_{p.o.}/AUC_{IndOH\ i.v.}$) × 100.

volume of distribution also increased significantly after IndOEt-NC dosing. Thanks to increased Cl_{TOT} and Vd_{SS}, IndOH half-life was not significantly altered after IndOEt-NC administration (Table 1).

Only IndOH was quantified in plasma after IndOEt-NC oral dosing (Fig. 2) suggesting a fast IndOEt release from nanocapsules and hydrolysis. Both IndOH profiles, after drug or IndOEt-NC administration, weighed 1/concentration, were described by one compartment open model.

IndOH non-compartmental pharmacokinetic parameters (Table 2) are in agreement with those reported for IndOH 5 mg/kg oral administration to rats (Ammoury et al., 1993). The comparison of Cp_{MAX}, t_{MAX}, $AUC_{0-\infty}$ and k_a determined after IndOH and IndOEt-NC oral dosing (Table 2) showed that the rate and the extent of IndOH absorption after the ester administration were lower than those observed for IndOH itself. The resulting bioavailabilities obtained for IndOH (77%) and for IndOEt-NC (65%) corroborate this observation. These differences in absorption rate are probably due to the time needed for IndOEt to be released from the nanocapsules and hydrolyzed. The absence of IndOEt in plasma suggests that it is released from nanocapsules and hydrolyzed in the intestine, in the liver and/or by the plasma esterases.

The results presented in this paper showed that 4 h after dosing, plasma levels of IndOH were 26% lower when IndOEt-NC was administered in comparison with the levels observed after IndOH dosing. These results explain the 29% lower antiedematogenic activity of IndOEt-NC after administration of the same dose as IndOH (Cruz et al., 2006a). Assuming direct link between plasma levels and effect, this observation confirms that IndOH is the only entity responsible for the antiedematogenic effect following IndOEt-NC administration. Although IndOEt *in vitro* was reported to be a COX inhibitor almost 700 times more selective to COX-2 (Kalgutkar et al., 2000), its nanoencapsulation within polymeric nanocapsules was not able to target the pro-drug to the site of action and the antiedematogenic effect observed was exclusively due the metabolite formed *in vivo*, i.e. IndOH.

In conclusion, IndOEt *in vivo* is rapidly released from nanocapsules and converted to IndOH independently of the administration route. Contrary to *in vitro* results, nanocapsules where not able to protect the ester and target the pro-drug to the biophase rendering IndOH the entity responsible for the antiedematogenic activity after IndOEt-NC dosing. Whether the formation of IndOH after oral dosing takes place in the intestine lumen, intestine wall or after the particles reach the blood stream remains to be investigated.

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